

SAMOA JOINT CANNERY OUTFALL

2002 Tradewind Season

EFFLUENT BIOASSAY TEST RESULTS

August 2002 Sampling



14 November 2002



CH2MHILL

TECHNICAL MEMORANDUM

BIOASSAY TESTING – JOINT CANNERY OUTFALL EFFLUENT AUGUST 2002 SAMPLING

Prepared For: StarKist Samoa (NPDES Permit AS0000019)
COS Samoa Packing (NPDES Permit AS0000027)

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Date: 14 November 2002

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United States Environmental Protection Agency, Region 9
Peter Peshut
American Samoa Environmental Protection Agency

Purpose

This memorandum presents the results of the bioassay testing of the Joint Cannery Outfall effluent sample that was collected in August 2002. The testing is required by the NPDES Permits that became effective in January 2001. This is the fourth required semiannual test required by the current permits and the eighteenth semiannual test conducted since testing for the Joint Cannery Outfall began in 1993.

Study Objectives

Section D.1 of the StarKist Samoa and COS Samoa Packing NPDES Permits requires that semiannual definitive acute bioassays (96-hour static bioassays) be conducted on the cannery effluent. The purpose of these tests is to determine whether, and at what effluent concentration, acute toxicity may be detected for the combined joint cannery effluent discharge into Pago Pago Harbor.

Study Approach

U.S. EPA has conducted a number of reviews of the effluent sampling, analysis, and bioassay tests conducted in the past. All comments from U.S. EPA have been incorporated into the sampling and sample handling standard operating procedures (SOP) or have been incorporated into the procedures used by the laboratory doing the test. The comments, responses, and SOP have been documented in previous reports.

The permit conditions require that the bioassay tests be conducted with the white shrimp, *Penaeus vannamei* (postlarvae). In the event *Penaeus vannamei* is not available at the time of the tests, the permit specifies the substitute species, *Mysidopsis bahia*, which now has been renamed *Americamysis bahia*. For the August 2002 sampling, *Penaeus vannamei* was not available and *Americamysis bahia* was used.

Effluent samples were collected from the StarKist Samoa and COS Samoa Packing facilities as 24-hour composite samples. The acute effluent bioassay test was conducted using a combined, flow-weighted, composite effluent sample made up from the effluent samples from both canneries, as allowed by the permit condition. This combined effluent bioassay is representative of the wastewater discharged from the joint cannery outfall to Pago Pago Harbor.

Effluent Sampling Methods

Between 0900 on 22 August 2002 and 0600 on 23 August 2002, 24-hour flow-weighted composite samples of final effluent were collected from both the StarKist Samoa and COS Samoa Packing effluent discharges. Samples were collected from the established effluent sampling sites. Detailed sampling procedures are described in the established SOP for cannery effluent sampling.

A total of eight grab samples were collected into pre-cleaned 1-gallon plastic cubitainers at each plant. Samples were collected at approximately three-hour intervals over a 24-hour period. The samples were stored on ice until the completion of the 24-hour sampling period. After all samples were collected a flow-proportioned composite sample was prepared. The grab sample collection times, effluent flow rates, and the relative effluent flow volumes calculated from plant flow records are summarized in Table 1. The relative effluent flow volumes were used to prepare the final composite sample, which was used to fill the sample container shipped to the laboratory for testing.

A 5-gallon cubitainer containing the composite sample was packed on ice in an ice chest for shipment to the laboratory. A chain-of-custody form for the sample was completed and sealed into a zip-lock bag and taped inside the lid of the ice chest. The sample was shipped via DHL to the testing laboratory. The chain-of-custody form is provided in Attachment I.

Table 1 StarKist Samoa and COS Samoa Packing 24-hour Composite Effluent Sample for Bioassay Testing August 2002						
Grab Sample Number	COS Samoa Packing		StarKist Samoa		COS Samoa Packing Percent of Total Flow	StarKist Samoa Percent Of Total Flow
	Sampling Date and Time	Effluent Flow Rate (mgd)	Sampling Date and Time	Effluent Flow Rate (mgd)		
1	22 Aug 2002 0900	0.88	22 Aug 2002 0900	2.060	3.5	8.2
2	1200	0.74	1200	2.260	2.9	9.0
3	1500	0.76	1500	2.820	3.0	11.2
4	1800	0.72	1800	2.510	2.9	10.0
5	2100	0.68	2100	2.450	2.7	9.8
6	23 Aug 2002 0000	0.68	23 Aug 2002 0000	2.600	2.7	10.4
7	0300	0.68	0300	2.500	2.7	10.0
8	0600	0.68	0600	2.090	2.7	8.3
Total		5.82		19.29	23.2	76.8
Mean		0.73		2.411	100.0	

Bioassay Testing Procedures

EnviroSystems, Inc. located in Hampton, New Hampshire conducted the bioassay tests. The testing procedures and results of the bioassay tests are provided in the Laboratory report included as Attachment II. This report summarizes the 96-hour acute bioassay test conducted with reference to U.S. EPA documents Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms (EPA/600/4-90/027F), August 1993 as the sources of methods for conducting the test. The bioassay test was conducted considering and including U.S. EPA's comments on previous bioassay tests, as documented in previous reports.

The test organisms were ≤ 5 days old and the test temperature was to be held at $20 \pm 1^\circ\text{C}$, with actual temperatures ranging between 20°C and 25°C . Salinity was

adjusted to 26 ppt at the start of the test and ranged between 25 and 26 ppt. Demonstrated potential for a lethal immediate dissolved oxygen demand (IDOD) had been discussed and documented in previous bioassay technical memoranda which describe the first two tests conducted in 1993. Therefore, all of the bioassay test chambers were continuously aerated during the bioassay tests to maintain adequate levels of dissolved oxygen (DO). The test was renewed with oxygenated sample at 48 hours.

Bioassay tests were carried out for effluent concentrations of 50, 25, 12.5, 6.25, and 3.1% as vol:vol dilutions in seawater. Water quality was monitored daily and parameters measured included DO, pH, salinity, and temperature. Total residual chlorine and ammonia were measured. Reference toxicant tests using sodium dodecyl sulfonate (SDS) are conducted regularly by ESI with the last one completed on August 9, 2002 and results were within one standard deviation of the historic laboratory mean.

Summary Results: Americamysis bahia Effluent Bioassay

All results from the bioassay tests are included in Attachment II. The results of the mysid bioassay tests indicate the 96-hour LC₅₀ for the effluent tested was 10.23 percent. The no observable effects concentration (NOEC) for the 96-hour bioassay was 6.25 percent and the least observable effects concentration (LOEC) was 12.5 percent. Results on a daily basis are summarized in Table 2.

Table 2 StarKist Samoa and COS Samoa Packing Combined Effluent Bioassay Results August 2002 Sampling			
Exposure Time	Parameter		
	LC ₅₀	NOEC	LOEC
24 hours	11.47%	6.25%	12.5%
48 hours	11.19%	6.25%	12.5%
72 hours	11.19%	6.25%	12.5%
96 hours	10.23%	6.25%	12.5%

Discussion

Table 3 summarizes the results of the effluent bioassay tests for the samples collected in the August 2002 sampling compared to the previous bioassay tests. The

LC₅₀, NOEC and LOEC are within the range obtained from previous reports where *Mysidopsis bahia* was used in place of *Penaeus vannamei*.

Conclusions

The bioassay tests for the Joint Cannery Outfall effluent for August 2002 do not indicate effluent toxicity levels to be of concern. As discussed in the previous bioassay test reports on the effluent, the time scale of the mixing of the effluent with the receiving water is on the order of minutes to seconds to achieve dilutions that will eliminate possible toxic effects as reflected by the bioassay results. For example, an LC₅₀ of 10.2%, which was observed in August 2002, corresponds to a dilution of 9.8:1, which is achieved within two seconds and within 3 meters of the discharge point. The discharge is located in about 180 feet of water and the effluent toxicity tests indicate that the discharge is diluted to non-toxic levels immediately after discharge and well within the initial dilution plume.

Table 3
StarKist Samoa and COS Samoa Packing
Combined Effluent Bioassay Results

Date	Species	Parameters		
		LC 50	NOEC	LOEC
2/93	<i>Penaeus vannamei</i>	4.8% ¹	3.1%	6.25%
10/93	<i>Penaeus vannamei</i>	15.67%	3.1%	6.25%
2/94	<i>Penaeus vannamei</i>	15.76%	<1.6%	1.6%
10/94	<i>Mysidopsis bahia</i> ²	31.2%	25%	50%
3/95	<i>Penaeus vannamei</i>	14.8%	6.25%	12.5%
3/95	<i>Mysidopsis bahia</i> ³	10.8%	6.25%	12.5%
2/96	<i>Penaeus vannamei</i>	>50%	>50%	>50%
2/96	<i>Mysidopsis bahia</i> ³	28.36%	12.5%	25%
3/96	<i>Penaeus vannamei</i>	44.4%	25%	50%
11/96	<i>Penaeus vannamei</i>	7.11%	3.1%	6.25%
03/97	<i>Penaeus vannamei</i>	39.36%	12.5%	25%
09/97	<i>Penaeus vannamei</i> ⁴	12.3%	6.25%	12.5%
06/98	<i>Mysidopsis bahia</i> ²	17.2%	6.25%	12.5%
11/98	<i>Mysidopsis bahia</i> ²	15%	6.25%	12.5%
02/00	<i>Mysidopsis bahia</i> ²	20%	6.25%	12.5%
08/00	<i>Mysidopsis bahia</i> ²	17.1%	3.1%	6.25%
03/01	<i>Americamysis bahia</i> ⁵	13.8%	12.5%	25%
10/01	<i>Americamysis bahia</i> ⁶	37.5%	25.0%	50.0%
3/02	<i>Americamysis bahia</i> ⁶	16.1%	12.5%	25%
8/02	<i>Americamysis bahia</i> ⁶	10.23%	6.25%	12.5%

¹The February 1993 samples were not aerated until after the first day of the test. For subsequent tests the samples were aerated for the entire duration of the tests.

²*Mysidopsis bahia* used as substitutes because *Penaeus vannamei* not available: as directed and approved by U. S. EPA.

³*Mysidopsis bahia* used in addition to *Penaeus vannamei* as described in text of technical memorandums reporting test results. Only one species is required by the permit conditions.

⁴Stage 1 (3 mm) *Penaeus vannamei* were used for testing because older Stage 7 and 8 (8-10 mm) *Penaeus vannamei* were not available.

⁵*Mysidopsis bahia* renamed *Americamysis bahia*. Results indicate increased toxicity because of low DO in renewal concentrations as renewal water was not aerated prior to use

⁶ *Mysidopsis bahia* renamed *Americamysis bahia*

ATTACHMENT I

Chain-of-Custody

CH2MHILL Analytical Services
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AND AGREEMENT TO PERFORM SERVICES

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Montgomery, AL 36116-1622
(334) 271-1444 FAX (334) 271-3428

☐ LRD 5090 Caterpillar Road
Redding, CA 96003-1412
(916) 244-5227 FAX (916) 244-4109

☐ LKW Canviro Analytical Laboratories, Inc.
50 Bathurst, Unit 12, Waterloo, Ontario, Canada N2V 2C5
(519) 747-2575 FAX (519) 747-3806

☐ CVD 2300 NW Walnut Boulevard
Corvallis, OR 97330-3638
(541) 752-4271 FAX (541) 752-0276

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Project Name SAMOA JOINT CANNERY OUTFALL																																																	
Company Name CH2M HILL																																																	
Project Manager or Contact & Phone # STEVE COSTA 707-677-0123										Report Copy to: SAME																																							
Requested Completion Date:					Site ID					Sample Disposal: Dispose <input checked="" type="checkbox"/> Return <input type="checkbox"/>																																							
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Relinquished By					Empty Bottles					Date/Time					Received By					Empty Bottles					Date/Time																								
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ATTACHMENT II

EnviroSystems, Inc. Laboratory Report

**TOXICOLOGICAL EVALUATION
OF A TREATED EFFLUENT:
BIOMONITORING SUPPORT FOR A NPDES PERMIT
AUGUST 2002**

American Samoa Joint Cannery Outfall

Prepared For

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August 2002
Reference Number CH2M10562-02-08

STUDY NUMBER 10562

EXECUTIVE SUMMARY

The following summarizes the results of acute exposure bioassays performed from August 26-30, 2002 in support of the NPDES biomonitoring requirements of the American Samoa Joint Cannery Outfall. The 96 hour acute definitive assays were conducted using the marine species *Americamysis bahia*.

Acute Toxicity Evaluation				
Species	Exposure	LC-50	NOEC	LOEC
<i>Americamysis bahia</i>	24-Hours	11.5%	6.25%	12.5%
	48-Hours	11.2%	6.25%	12.5%
	72-Hours	11.2%	6.25%	12.5%
	96-Hours	10.2%	6.25%	12.5%

**TOXICOLOGICAL EVALUATION
OF A TREATED EFFLUENT:
BIOMONITORING SUPPORT FOR A NPDES PERMIT
AUGUST 2002**

American Samoa Joint Cannery Outfall

1.0 INTRODUCTION

Acute toxicity tests involve preparing a series of concentrations by diluting effluent with control water. Groups of test organisms are exposed to each effluent concentration and a control for a specified period. In acute tests, mortality data for each concentration are used to calculate (by regression) the median lethal concentration, or LC-50, defined as the effluent concentration which kills half of the test organisms. Samples with high LC-50 values are less likely to cause significant environmental impact. The acute no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) document the highest and lowest effluent concentrations that have no impact and a significant impact on the test species, respectively.

This report presents the results of acute toxicity tests conducted on an effluent sample collected from the American Samoa Joint Cannery Outfall. Testing was based on programs and protocols developed by the US EPA (1993) and involved conducting 96 hour acute static renewal toxicity tests with the marine species *Americamysis bahia*. Testing was performed at EnviroSystems, Incorporated (ESI), Hampton, New Hampshire in accordance with the provisions of the NELAC Standards (2000).

2.0 MATERIALS AND METHODS

2.1 General Methods

Toxicological and analytical protocols used in this program follow procedures primarily designed by the EPA to provide standard approaches for the evaluation of toxicological effects of discharges on aquatic organisms, and for the analysis of water samples. See Section 4.0 for a list of references.

2.2 Test Species

A. bahia, ≤ 5 days old, were from cultures maintained by ESI. Test organisms were transferred to test chambers by large bore pipet, minimizing the amount of water added to test solutions.

2.3 Effluent and Dilution Water

The effluent sample used in the assay was identified as "02-TW." Sample collection information is provided in Table 4. Upon receipt, the sample was stored at 4°C. All sample material used in the assay was warmed to 20±1°C prior to preparing test solutions. Total residual chlorine (TRC) was measured using amperometric titration (MDL 0.05 mg/L). As the effluent sample contained <0.05 mg/L, TRC dechlorination with sodium thiosulfate was not required (EPA 1993). Aliquots of the undiluted effluent sample were collected for ammonia analysis when the sample arrived and again prior to renewal. At arrival, the effluent sample had a salinity of 13‰. Salinity of the effluent was increased to 25‰ by the addition of artificial sea salts. Test concentrations for the assays were 50%, 25%, 12.5%, 6.25% and 3.1% effluent with a dilution water control.

The dilution water used in this assay was collected by ESI from its sea water system. The water is pumped from the Hampton Estuary on the flood tide, filtered through a high volume sand filter, and stored in 3000 gallon polyethylene tanks. The water is classified as Class SA-1 by the State of New Hampshire and has been used for culture of test organisms for over 20 years. Sea water used in the assay had a salinity of 26‰ and a TRC of <0.05 mg/L.

2.4 Acute Toxicity Tests

The 96 hour acute static renewal toxicity test was conducted at 20±1°C with a photoperiod of 16:8 hours light:dark. Test chambers for the acute assays were 250 mL glass beakers containing 200 mL test solution in each of 5 replicates, with 10 organisms/replicate. Survival and dissolved oxygen were measured daily in all replicates. Salinity, pH and temperature were measured daily in one replicate of each test treatment. Test solutions were renewed after 48 hours using effluent from the start sample. Mysid shrimp were fed daily with <24 hour old brine shrimp.

2.5 Data Analysis

At 24 hour intervals, survival data was analyzed to assess toxicity using a program developed by Stephan (1982). LC-50 values were computed using the Spearman-Kärber and Probit computation methods. If survival in the highest test concentration was >50%, LC-50 values were obtained by direct observation of the raw data. The NOEC was determined as the highest test concentration which caused no significant mortality while the LOEC was determined as the lowest concentration that did cause significant mortality.

2.6 Quality Control

As part of the laboratory quality control program, standard reference toxicant assays are conducted on a regular basis for each test species. These results provide relative health and response data while allowing for comparison with historic data sets. A forty-eight hour acute reference toxicant assay was performed with *A. bahia* on August 9, 2002. Results of this assay were within one standard deviation of the historic mean for the species. See Table 2 for details.

3.0 RESULTS

Results of the acute exposure bioassay conducted using the mysid shrimp are summarized in Table 1. A summary of reference toxicant data for the test species is presented in Table 2. Effluent and dilution water characteristics are presented in Table 3. Sample collection information is provided in Table 4. Table 5 provides a summary of historic data associated with the discharge. Support data are included in Appendix A.

3.1 Acute Toxicity Test - *Americamysis bahia*

Survival in laboratory diluent control was 100% after 24 hours, 98% after 48 hours, 96% after 72 hours, and 94% after 96 hours exposure. These results are an indication of healthy test organisms and that the dilution water had no adverse impact on the outcome of the assay.

Table 1 provides a summary of the acute exposure data and results.

3.2 Summary

The salinity adjusted effluent sample for the American Samoa Joint Cannery site exhibited signs of acute toxicity to the mysid shrimp, *Americamysis bahia*, during the 96 hour exposure period.

4.0 LITERATURE CITED

- APHA. 1998. *Standard Methods for the Examination of Water and Wastewater*, 20th Edition. Washington D.C.
- Stephan, C. 1982. Documentation for Computing LC-50 Values with a Mini Computer. Unpublished.
- US EPA. 1993. *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms*. Fourth Edition. EPA/600/4-90/027F.
- US EPA. 2001. *Attachment G: NPDES Whole Effluent Toxicity Testing, Monitoring and Reporting Tips and Common Pitfalls*. Dated December 2001. US EPA Region I Offices, Boston, Massachusetts.
- National Environmental Laboratory Accreditation Conference: Quality Systems*. Chapter 5. June 2000.

TABLE 1. Summary of Acute Evaluation Results. American Samoa Joint Cannery Outfall Effluent Evaluation. August 2002.

Concentration % Effluent	Exposure	Replicates					Mean	Standard Deviation	Coefficient of Variation
		A	B	C	D	E			
Lab Control	Start	10	10	10	10	10	100%	0.000	0.00%
	24-Hours	10	10	10	10	10	100%	0.000	0.00%
	48-Hours	10	9	10	10	10	98%	0.447	4.56%
	72 Hours	10	8	10	10	10	96%	0.894	9.32%
	96-Hours	9	8	10	10	10	94%	0.894	9.52%
3.1%	24-Hours	10	10	10	10	10	100%	0.000	0.00%
	48-Hours	10	10	10	10	10	100%	0.000	0.00%
	72 Hours	10	10	10	10	10	100%	0.000	0.00%
	96-Hours	10	10	10	10	9	98%	0.447	4.56%
6.25%	24-Hours	10	10	9	10	10	98%	0.447	4.56%
	48-Hours	10	10	9	10	10	98%	0.447	4.56%
	72 Hours	10	10	9	10	10	98%	0.447	4.56%
	96-Hours	10	10	9	10	10	98%	0.447	4.56%
12.5%	24-Hours	2	7	5	2	2	36%	2.302	63.95%
	48-Hours	2	6	4	2	2	32%	1.789	55.90%
	72 Hours	2	6	4	2	2	32%	1.789	55.90%
	96-Hours	2	3	4	2	2	26%	0.894	34.40%
25%	24-Hours	0	0	0	1	0	2%	0.447	223.61%
	48-Hours	0	0	0	1	0	2%	0.447	223.61%
	72 Hours	0	0	0	1	0	2%	0.447	223.61%
	96-Hours	0	0	0	1	0	2%	0.447	223.61%
50%	24-Hours	0	0	0	0	0	0%	0.000	-
	48-Hours	0	0	0	0	0	0%	0.000	-
	72 Hours	0	0	0	0	0	0%	0.000	-
	96-Hours	0	0	0	0	0	0%	0.000	-

SUMMARY OF ENDPOINTS

Exposure Period	LC-50 (95% Limits)	METHOD	NOEC	LOEC
24 Hours	11.47 (10.35-12.71)	Probit	6.25%	12.5%
48 Hours	11.19 (10.10-12.39)	Probit	6.25%	12.5%
72 Hours	11.19 (10.10-12.39)	Probit	6.25%	12.5%
96 Hours	10.23 (9.23-11.34)	Spearman-Kärber	6.25%	12.5%

TABLE 2. Summary of Reference Toxicant Data. American Samoa Joint Cannery Outfall Effluent Evaluation. August 2002.

Concentrations Expressed as mg/L Sodium Dodecyl Sulfate

Species	Date	LC-50	Historic Mean	Number of Tests	±1 STD Deviation	±2 STD Deviation
<i>A. bahia</i>	08/09/02	22.0	19.8	139	4.31	8.63

TABLE 3. Summary of Effluent and Diluent Characteristics. American Samoa Joint Cannery Outfall Effluent Evaluation. August 2002.

Parameter	Units	EFFLUENT	DILUENT
Salinity - on Arrival	‰	13	26
After Salinity Adjustment ‡	‰	26	-
pH - on Arrival	SU	6.53	7.80
After Salinity Adjustment ‡	SU	7.19	-
TRC	mg/L	<0.05	<0.05
Dissolved Oxygen	mg/L	1.5	7.3
Ammonia - at Start	mg/L as N	35.60	<0.10
Unionized Ammonia	mg/L as N	0.048	-
Ammonia - at Start- Salinity Adjusted ‡	mg/L as N	18.43	-
Unionized Ammonia ‡	mg/L as N	0.11	-
Ammonia - at 48 Hours	mg/L as N	38.05	<0.10

TABLE 4. Summary of Sample Collection Information. American Samoa Joint Cannery Outfall Effluent Evaluation. August 2002.

Sample Description	Type	Collection		Receipt		Arrival Temp °C
		Date	Time	Date	Time	
EFFLUENT	Comp	08/23/02	ND	08/26/02	1515	17

COMMENTS:

‡ - Analysis performed on 50% effluent, the highest concentration tested.

ND - No data recorded on chain of custody.

TABLE 5. Summary of Starkist Samoa and COS Samoa Packing Combined Effluent Bioassay Results. American Samoa Joint Cannery Outfall Effluent Evaluation. August 2002.

Date	Species	96-Hour Endpoints		
		LC-50	NOEC	LOEC
02/93 ¹	<i>Penaeus vannamei</i>	4.8%	3.1%	6.25%
10/93 ¹	<i>Penaeus vannamei</i>	15.67%	3.1%	6.25%
02/94 ¹	<i>Penaeus vannamei</i>	15.76%	<1.6%	1.6%
10/94 ¹	<i>Americamysis bahia</i>	31.2%	25.0%	50.0%
03/95 ¹	<i>Penaeus vannamei</i>	14.8%	6.25%	12.5%
03/95 ¹	<i>Americamysis bahia</i>	10.8%	6.25%	12.5%
02/96 ¹	<i>Penaeus vannamei</i>	>50.0%	>50.0%	>50.0%
03/96 ¹	<i>Penaeus vannamei</i>	44.4%	25.0%	50.0%
11/96 ¹	<i>Penaeus vannamei</i>	7.11%	3.1%	6.25%
03/97 ¹	<i>Penaeus vannamei</i>	39.36%	12.5%	25.0%
09/97 ¹	<i>Penaeus vannamei</i>	12.3%	6.25%	12.5%
06/98 ¹	<i>Americamysis bahia</i>	17.2%	6.25%	12.5%
11/98 ¹	<i>Americamysis bahia</i>	15.0%	6.25%	12.5%
02/00 ¹	<i>Americamysis bahia</i>	20.0%	6.25%	12.5%
08/00 ¹	<i>Americamysis bahia</i>	17.1%	3.1%	6.25%
03/01 ²	<i>Americamysis bahia</i>	13.81%	12.5%	25.0%
03/02 ²	<i>Americamysis bahia</i>	16.13%	12.5%	25.0%
08/02 ²	<i>Americamysis bahia</i>	10.23%	6.25%	12.5%

Notes:

- ¹ Assays conducted by Advanced Biological Testing, Inc., Rohnert Park, California
- ² Assay conducted by EnviroSystems, Inc., Hampton, New Hampshire

APPENDIX A
DATA SHEETS
STATISTICAL SUPPORT

Contents	Number of Pages
Methods Used in NPDES Permit Biomonitoring Testing	1
<i>A. bahia</i> Acute Bioassay Data Summary	2
<i>A. bahia</i> Survival Statistics: LC-50, NOEC	16
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Dilution Preparation Log	1
Record of Meters Used for Water Quality Measurements	1
Sample Receipt Record	1
Chain of Custody	1

METHODS USED IN NPDES PERMIT BIOMONITORING TESTING

Parameter	Method
Acute Exposure Bioassays	
<i>Ceriodaphnia dubia</i> , <i>Daphnia pulex</i>	EPA 600/4-90/027
<i>Pimephales promelas</i>	EPA 600/4-90/027
<i>Americamysis bahia</i>	EPA 600/4-90/027
<i>Menidia beryllina</i> , <i>Cyprinodon variegatus</i>	EPA 600/4-90/027
Chronic Exposure Bioassays	
<i>Ceriodaphnia dubia</i>	EPA 600/4-91/002, 1002.0
<i>Pimephales promelas</i>	EPA 600/4-91/002, 1000.0
<i>Cyprinodon variegatus</i>	EPA 600/4-91/003, 1004.0
<i>Menidia beryllina</i>	EPA 600/4-91/003, 1006.0
<i>Arbacia punctulata</i>	EPA 600/4-91/003, 1008.0
<i>Champia parvula</i>	EPA 600/4-91/003, 1009.0
Trace Metals:	
ICP Metals	EPA 200.7/SW 6010
Hardness	Standard Methods 20 th Edition - Method 2340 B
Wet Chemistries:	
Alkalinity	EPA 310.2
Chlorine, Residual	Standard Methods 20 th Edition - Method 4500CLD
Total Organic Carbon	Standard Methods 20 th Edition - Method 5310.6
Specific Conductance	Standard Methods 20 th Edition - Method 2510B
Nitrogen - Ammonia	Standard Methods 20 th Edition - Method 4500NH3G
pH	Standard Methods 20 th Edition - Method 4500H+B
Solids, Total (TS)	Standard Methods 20 th Edition - Method 2540.B
Solids, Total Suspended (TSS)	Standard Methods 20 th Edition - Method 2540D
Dissolved Oxygen	Standard Methods 20 th Edition - Method 4500-O G

ACUTE BIOASSAY DATA SUMMARY

STUDY: 10562		SAMPLE RECEIVED: 8/20/02		"AS RECEIVED" EFFLUENT AND DILUENT CHEMISTRIES							
CLIENT: CH2M Hill		TEST ORGANISM: A. bahia		TRC	AMM 0 HR*	AMM 48 HR*	pH	DO	Salinity		
SAMPLE: American Samoa		ORGANISM SUPPLIER: EST		EFFLUENT	40.05	AP	RC	6.53	1.5	13	
DILUENT: LAB SALT		ORGANISM BATCH/AGE: 082602 AP / 5 DAY		DILUENT	40.05	AP	RC	7.80	7.3	26	

SALINITY ADJUSTMENT RECORD (IF APPLICABLE): 2000 ML EFFLUENT + 28 G SEA SALTS = 100% ACTUAL PERCENTAGE

CONC	REP	SURVIVAL					DISSOLVED OXYGEN (MG/L)†					PH (SU)					TEMPERATURE (°C)					SALINITY (ppt)						
		0	24	48	72	96	0	24	48*	48*	72	96	0	24	48*	48*	72	96	0	24	48*	48*	72	96				
LAB	A	10	10	10	10	9	7.3	7.1	6.4	6.5	7.6	6.5	7.80	7.94	8.08	8.07	7.67	8.01	20	24	24	23	25	24	26	26	25	25
	B	10	10	9	8	8	7.2	7.2	6.4	6.6	6.6	6.5																
	C	10	10	10	10	10	7.2	7.2	6.5	6.7	6.7	6.4																
	D	10	10	10	10	10	7.2	7.0	6.4	6.7	6.6	6.4																
	E	10	10	10	10	10	7.2	6.8	6.4	6.7	6.6	6.4																
3.1%	A	10	10	10	10	10	7.9	6.0	6.4	6.6	6.4	6.4	7.75	7.74	8.09	8.02	8.07	8.01	20	24	24	23	24	25	26	26	27	
	B	10	10	10	10	10	7.0	6.5	6.5	6.6	6.6	6.5																
	C	10	10	10	10	10	7.1	6.8	6.3	6.6	6.7	6.4																
	D	10	10	10	10	10	7.1	6.5	6.3	6.5	6.7	6.5																
	E	10	10	10	10	9	7.1	6.6	6.3	6.3	6.5	6.4																
6.25%	A	10	10	10	10	10	7.1	6.8	6.2	6.4	6.6	6.4	7.69	7.63	8.04	7.92	8.12	8.04	20	24	24	23	24	24	26	26	27	
	B	10	10	10	10	10	7.1	6.9	6.2	6.4	6.6	6.4																
	C	10	9	9	9	9	7.1	7.2	6.4	6.5	6.7	6.3																
	D	10	10	10	10	10	7.1	7.1	6.4	6.6	6.5	6.4																
	E	10	10	10	10	10	7.1	7.3	6.3	6.6	6.4	6.2																
DATE	8/20	8/27	8/28	8/29	8/30	8/20	8/27	8/28	8/28	8/29	8/30	8/27	8/28	8/28	8/29	8/30	8/27	8/28	8/28	8/29	8/30	8/27	8/28	8/28	8/29	8/30		
TIME	1700	1620	1630	1610	1513	1615	1615	1615	1645	1605	1506	1615	1615	1645	1605	1506	1615	1615	1645	1605	1506	1615	1615	1645	1605	1506		
INITIALS	RC	RC	RC	PS	LM	AP	RC	RC	RC	PS	LM	RC	RC	RC	PS	LM	RC	RC	RC	PS	LM	RC	RC	RC	PS	LM		
FED?	Y	Y	Y	Y		Y	Y	Y	Y	Y		Y	Y	Y	Y		Y	Y	Y	Y		Y	Y	Y	Y			

* - Pull on 50% effluent also.

◊ - "Old" water qualities (prior to renewal)

† - AERATE FROM START!

☆ - "New" water qualities (post renewal)

ACUTE BIOASSAY DATA SUMMARY

STUDY: 10562		SAMPLE RECEIVED: 8/24/02		"AS RECEIVED" EFFLUENT AND DILUENT CHEMISTRIES													
CLIENT: CH2M Hill		TEST ORGANISM: <i>A. bahia</i>		TRC		AMM 0 HR*		AMM 48 HR*		pH		DO		Salinity			
SAMPLE: American Samoa		ORGANISM SUPPLIER:		EFFLUENT		See page 1 for details and salinity adjustment record.											
DILUENT: LAB SALT		ORGANISM BATCH/AGE:		DILUENT													

CONC	REP	SURVIVAL					+ DISSOLVED OXYGEN (MG/L)†					PH (SU)					TEMPERATURE (°C)					SALINITY (ppt)							
		0	24	48	72	96	0	24	48*	48☆	72	96	0	24	48*	48☆	72	96	0	24	48*	48☆	72						
12.5%	A	10	2	2	2	2	6.8	7.1	6.0	6.4	6.4	6.3	7.57	7.57	8.04	7.74	8.11	8.12	20	24	24	23	24	24	26	26	27	25	27
	B	10	7	6	6	3	6.8	5.1	5.8	5.8	6.5	6.1																	
	C	10	5	4	4	4	6.9	7.2	6.0	5.9	6.3	6.2																	
	D	10	2	2	2	2	6.9	7.0	6.0	6.0	6.4	6.1																	
	E	10	2	2	2	2	6.8	7.4	6.1	6.1	6.4	6.2																	
25%	A	10	—	—	—	—	6.9	7.2	—	—	—	—	7.38	7.71	8.11	7.48	7.78	8.19	20	24	24	23	25	24	26	26	27	25	26
	B	10	—	—	—	—	6.8	6.4	—	—	—	—																	
	C	10	—	—	—	—	6.7	6.2	—	—	—	—																	
	D	10	1	1	1	1	6.7	6.8	6.1	5.3	5.9	6.2																	
	E	10	—	—	—	—	6.7	7.0	—	—	—	—																	
50%	A	10	—	—	—	—	5.7	4.9	—	—	—	—	7.19	7.81	—	—	—	—	20	24	—	—	—	—	25	26	—	—	—
	B	10	—	—	—	—	5.8	7.8	—	—	—	—																	
	C	10	—	—	—	—	5.8	7.8	—	—	—	—																	
	D	10	—	—	—	—	5.8	7.0	—	—	—	—																	
	E	10	—	—	—	—	5.9	6.8	—	—	—	—																	
DATE	8/26	8/27	8/28	8/29	8/30	8/26	8/27	8/28	8/28	8/29	8/30	8/27	8/28	8/28	8/29	8/30	8/27	8/28	8/28	8/29	8/30	8/27	8/28	8/28	8/29	8/29	8/29	8/29	
TIME	1700	1630	1630	1610	1513	1555	1615	1615	1645	1605	1506	1615	1615	1645	1605	1306	1615	1615	1645	1605	1506	1615	1615	1645	1605	1605	1605	1605	
INITIALS	RC	RC	RC	PS	LM	AP	RC	RC	RC	PS	LM	RC	RC	RC	PS	LM	RC	RC	RC	PS	LM	RC	RC	RC	PS	PS	PS	PS	
FED?	Y	Y	Y	Y		✓	Y	Y	Y	Y		Y	Y	Y	Y		Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		

* - Pull on 50% effluent also.
 ◇ - "Old" water qualities (prior to renewal)

† - AERATE FROM START!
 ☆ - "New" water qualities (post renewal)

Title: 10562 American Samoa A. bahia Survival 24hrs
 File: 10562AmS24 Transform: ARC SINE(SQUARE ROOT(Y))

Chi-Square Test for Normality

Actual and Expected Frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.0100	7.2600	11.4600	7.2600	2.0100
OBSERVED	1	3	23	2	1

Chi-Square = 18.9462 (p-value = 0.0008)

Critical Chi-Square = 13.277 (alpha = 0.01 , df = 4)
 = 9.488 (alpha = 0.05 , df = 4)

Data FAIL normality test (alpha = 0.01). Try another transformation.

Warning - The first three homogeneity tests are sensitive to non-normality and should not be performed with this data as is.

Title: 10562 American Samoa A. bahia Survival 24hrs
 File: 10562AmS24 Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's Test for Normality

D = 0.2800
 W = 0.7715

Critical W = 0.9000 (alpha = 0.01 , N = 30)
 W = 0.9270 (alpha = 0.05 , N = 30)

Data FAIL normality test (alpha = 0.01). Try another transformation.

Warning - The first three homogeneity tests are sensitive to non-normality and should not be performed with this data as is.

Title: 10562 American Samoa A. bahia Survival 24hrs
 File: 10562AmS24 Transform: ARC SINE(SQUARE ROOT(Y))

Hartley's Test for Homogeneity of Variance
 Bartlett's Test for Homogeneity of Variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
 Additional transformations are useless.

Title: 10562 American Samoa A. bahia Survival 24hrs
 File: 10562AmS24 Transform: ARC SINE(SQUARE ROOT(Y))

Summary Statistics on Transformed Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Lab	5	1.4120	1.4120	1.4120
2	3.1	5	1.4120	1.4120	1.4120
3	6.25	5	1.2490	1.4120	1.3794
4	12.5	5	0.4636	0.9912	0.6335
5	25	5	0.1588	0.3218	0.1914
6	50	5	0.1588	0.1588	0.1588

Title: 10562 American Samoa A. bahia Survival 24hrs
 File: 10562AmS24 Transform: ARC SINE(SQUARE ROOT(Y))

Summary Statistics on Transformed Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Lab	0.0000	0.0000	0.0000	0.0000
2	3.1	0.0000	0.0000	0.0000	0.0000
3	6.25	0.0053	0.0729	0.0326	5.2837
4	12.5	0.0594	0.2437	0.1090	38.4674
5	25	0.0053	0.0729	0.0326	38.0838
6	50	0.0000	0.0000	0.0000	0.0000

Title: 10562 American Samoa A. bahia Survival 24hrs
 File: 10562AmS24 Transform: ARC SINE(SQUARE ROOT(Y))

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	Lab	1.4120				
2	3.1	1.4120	27.50	16.00	5.00	
3	6.25	1.3794	25.00	16.00	5.00	
4	12.5	0.6335	15.00	16.00	5.00	*
5	25	0.1914	15.00	16.00	5.00	*
6	50	0.1588	15.00	16.00	5.00	*

Critical values are 1 tailed (k = 5)

STUDY NO.: 10562 ASSAY START: 08/26/02

SPECIES: A. bahia EXPOSURE: 24hrs

SAMPLE: CH2MHill Am. Samoa Effluent

The binomial test shows that 6.25 and 25 can be used as statistically sound, conservative 95 percent confidence limits because the actual confidence level associated with these limits is greater than 95 percent.

An approximate LC50 of 11.00089 is obtained by nonlinear interpolation between 6.25 and 12.5

-----RESULTS CALCULATED USING THE MOVING AVERAGE METHOD-----

span	G	LC50	95 Percent Confidence Limits	
4	1.867084E-02	11.66674	10.26085	13.23047
3	.0215173	11.62337	10.34632	13.21626
2	2.510172E-02	11.55849	10.46717	12.71222
1	6.680606E-02	11.00088	10.00277	12.48365

An LC50 calculated using the Moving Average method may not be a very good estimate if the span is much less than the number of concentrations.

-----RESULTS CALCULATED USING THE PROBIT METHOD-----

Iterations	G	H	Chi-Square	Probability
5	7.691131E-02	1	1.214623	.7494993

Slope = 6.985039
95 Percent Confidence Limits = 5.047886 and 8.922192

LC50 = 11.46846
95 Percent Confidence Limits = 10.347 and 12.70672

-----RESULTS CALCULATED USING THE TRIMMED SPEARMAN-KARBER METHOD-----

Conc.	Dose = Ln (Conc)	Percent Dead	Monotonic Rel. Freq.	Trimmed Rel. Freq.
50	3.912023	100	1	1
25	3.218876	98	.98	.98
12.5	2.525729	64	.64	.64
6.25	1.832581	2	.02	.02
3.1	1.131402	0	0	0

Alpha = 0 %

Groups trimmed and therefore not used in estimating LC50 are marked with an asterisk above.

LC50 = 11.34308

Estimated 95 Percent Confidence Limits

Lower: 10.24478

Upper: 12.55911

Variance estimate = 2.592791E-03

STUDY NO.: 10562 ASSAY START: 08/26/02

SPECIES: A. bahia EXPOSURE: 24hrs

SAMPLE: CH2MHill Am. Samoa Effluent

SUMMARY TABLE

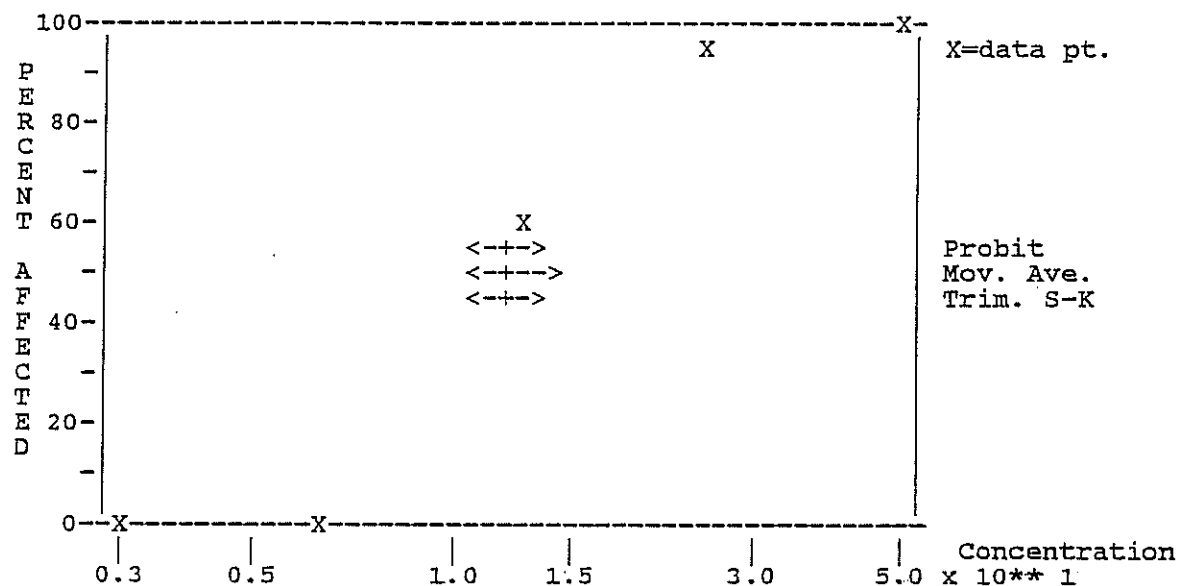
09-19-2002 09:58:45

Data:	Conc.	Exposed	Dead	Percent
	50	50	50	100
	25	50	49	98
	12.5	50	32	64
	6.25	50	1	2
	3.1	50	0	0

	LC50	Lower 95% Limit	Upper 95% Limit	
Probit Analysis	11.46846	10.347	12.70672	
Moving Average	11.66674	10.26085	13.23047	Span= 4
Spearman-Kärber	11.34308	10.24478	12.55911	Alpha= 0 %

Binomial 11.00089

Compare results with original data to see if they are reasonable.



Title: 10562 American Samoa A. bahia Survival 48hrs
File: 1056248AmS Transform: ARC SINE(SQUARE ROOT(Y))

Chi-Square Test for Normality

Actual and Expected Frequencies					
INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.0100	7.2600	11.4600	7.2600	2.0100
OBSERVED	2	3	23	0	2

Chi-Square = 21.3803 (p-value = 0.0003)

Critical Chi-Square = 13.277 (alpha = 0.01 , df = 4)
= 9.488 (alpha = 0.05 , df = 4)

Data FAIL normality test (alpha = 0.01). Try another transformation.

Warning - The first three homogeneity tests are sensitive to non-normality and should not be performed with this data as is.

Title: 10562 American Samoa A. bahia Survival 48hrs
File: 1056248AmS Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's Test for Normality

D = 0.2082
W = 0.8334

Critical W = 0.9000 (alpha = 0.01 , N = 30)
W = 0.9270 (alpha = 0.05 , N = 30)

Data FAIL normality test (alpha = 0.01). Try another transformation.

Warning - The first three homogeneity tests are sensitive to non-normality and should not be performed with this data as is.

Title: 10562 American Samoa A. bahia Survival 48hrs
File: 1056248AmS Transform: ARC SINE(SQUARE ROOT(Y))

Hartley's Test for Homogeneity of Variance
Bartlett's Test for Homogeneity of Variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

Title: 10562 American Samoa A. bahia Survival 48hrs
 File: 1056248AmS Transform: ARC SINE(SQUARE ROOT(Y))

Summary Statistics on Transformed Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Lab	5	1.2490	1.4120	1.3794
2	3.1	5	1.4120	1.4120	1.4120
3	6.25	5	1.2490	1.4120	1.3794
4	12.5	5	0.4636	0.8861	0.5923
5	25	5	0.1588	0.3218	0.1914
6	50	5	0.1588	0.1588	0.1588

Title: 10562 American Samoa A. bahia Survival 48hrs
 File: 1056248AmS Transform: ARC SINE(SQUARE ROOT(Y))

Summary Statistics on Transformed Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Lab	0.0053	0.0729	0.0326	5.2837
2	3.1	0.0000	0.0000	0.0000	0.0000
3	6.25	0.0053	0.0729	0.0326	5.2837
4	12.5	0.0361	0.1901	0.0850	32.0869
5	25	0.0053	0.0729	0.0326	38.0838
6	50	0.0000	0.0000	0.0000	0.0000

Title: 10562 American Samoa A. bahia Survival 48hrs
 File: 1056248AmS Transform: ARC SINE(SQUARE ROOT(Y))

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	Lab	1.3794				
2	3.1	1.4120	30.00	16.00	5.00	
3	6.25	1.3794	27.50	16.00	5.00	
4	12.5	0.5923	15.00	16.00	5.00	*
5	25	0.1914	15.00	16.00	5.00	*
6	50	0.1588	15.00	16.00	5.00	*

Critical values are 1 tailed (k = 5)

STUDY NO.: 10562 ASSAY START: 08/26/02

SPECIES: A. bahia EXPOSURE: 48 hours

SAMPLE: CH2MHill American Samoa

The binomial test shows that 6.25 and 12.5 can be used as statistically sound, conservative 95 percent confidence limits because the actual confidence level associated with these limits is greater than 95 percent.

An approximate LC50 of 10.6826 is obtained by nonlinear interpolation between 6.25 and 12.5

-----RESULTS CALCULATED USING THE MOVING AVERAGE METHOD-----

Span	G	LC50	95 Percent Confidence Limits	
1	1.867195E-02	11.43436	10.05015	12.96518
3	2.152512E-02	11.37471	10.13058	12.91414
5	2.510173E-02	11.2926	10.21447	12.41939
1	.0600481	10.6826	9.778212	11.9566

An LC50 calculated using the Moving Average method may not be a very good estimate if the span is much less than the number of concentrations.

-----RESULTS CALCULATED USING THE PROBIT METHOD-----

Iterations	G	H	Chi-Square	Probability
1	7.773012E-02	1	2.206272	.5307138

Slope = 7.092948
95 Percent Confidence Limits = 5.115425 and 9.07047

LC50 = 11.18858
95 Percent Confidence Limits = 10.10101 and 12.38541

-----RESULTS CALCULATED USING THE TRIMMED SPEARMAN-KARBER METHOD-----

Conc.	Dose = Ln (Conc)	Percent Dead	Monotonic Rel. Freq.	Trimmed Rel. Freq.
50	3.912023	100	1	1.125 *
15	3.218876	98	.98	1.1 *
12.5	2.525729	68	.68	.725
6.25	1.832581	2	.02	-9.999999E-02 *
1	1.131402	0	0	-.125 *

Alpha = 10 %

Groups trimmed and therefore not used in estimating LC50 are marked with an asterisk above.

LC50 = 10.74899

Estimated 95 Percent Confidence Limits

Lower: 9.607583 Upper: 12.02599

Variance estimate = 3.150509E-03

STUDY NO.: 10562 ASSAY START: 08/26/02

SPECIES: A. bahia EXPOSURE: 48 hours

SAMPLE: CH2MHill American Samoa

SUMMARY TABLE

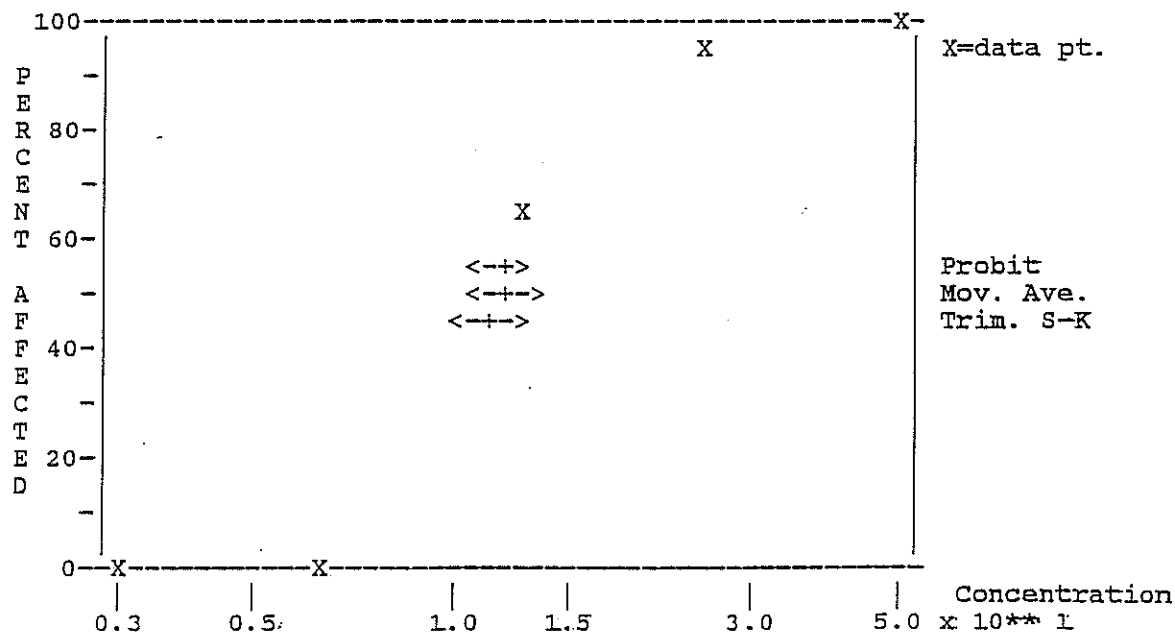
10-04-2002 10:53:44

Data:	Conc.	Exposed	Dead	Percent
	50	50	50	100
	25	50	49	98
	12.5	50	34	68
	6.25	50	1	2
	3.1	50	0	0

	LC50	Lower 95% Limit	Upper 95% Limit	
Probit Analysis	11.18858	10.10101	12.38541	
Moving Average	11.43436	10.05015	12.96518	Span= 4
Spearman-Kärber	10.74899	9.607583	12.02599	Alpha= 10 %

Binomial 10.6826

Compare results with original data to see if they are reasonable.



Title: 10562 American Samoa A. bahia Survival 72hrs
File: AmS72 Transform: ARC SINE(SQUARE ROOT(Y))

Chi-Square Test for Normality

Actual and Expected Frequencies					
INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.0100	7.2600	11.4600	7.2600	2.0100
OBSERVED	2	3	23	0	2

Chi-Square = 21.3803 (p-value = 0.0003)

Critical Chi-Square = 13.277 (alpha = 0.01 , df = 4)
= 9.488 (alpha = 0.05 , df = 4)

Data FAIL normality test (alpha = 0.01). Try another transformation.

Warning - The first three homogeneity tests are sensitive to non-normality and should not be performed with this data as is.

Title: 10562 American Samoa A. bahia Survival 72hrs
File: AmS72 Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's Test for Normality

D = 0.2614
W = 0.8998

Critical W = 0.9000 (alpha = 0.01 , N = 30)
W = 0.9270 (alpha = 0.05 , N = 30)

Data FAIL normality test (alpha = 0.01). Try another transformation.

Warning - The first three homogeneity tests are sensitive to non-normality and should not be performed with this data as is.

Title: 10562 American Samoa A. bahia Survival 72hrs
File: AmS72 Transform: ARC SINE(SQUARE ROOT(Y))

Hartley's Test for Homogeneity of Variance
Bartlett's Test for Homogeneity of Variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

Title: 10562 American Samoa A. bahia Survival 72hrs
 File: AmS72 Transform: ARC SINE(SQUARE ROOT(Y))

Summary Statistics on Transformed Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Lab	5	1.1071	1.4120	1.3510
2	3.1	5	1.4120	1.4120	1.4120
3	6.25	5	1.2490	1.4120	1.3794
4	12.5	5	0.4636	0.8861	0.5923
5	25	5	0.1588	0.3218	0.1914
6	50	5	0.1588	0.1588	0.1588

Title: 10562 American Samoa A. bahia Survival 72hrs
 File: AmS72 Transform: ARC SINE(SQUARE ROOT(Y))

Summary Statistics on Transformed Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Lab	0.0186	0.1363	0.0610	10.0916
2	3.1	0.0000	0.0000	0.0000	0.0000
3	6.25	0.0053	0.0729	0.0326	5.2837
4	12.5	0.0361	0.1901	0.0850	32.0869
5	25	0.0053	0.0729	0.0326	38.0838
6	50	0.0000	0.0000	0.0000	0.0000

Title: 10562 American Samoa A. bahia Survival 72hrs
 File: AmS72 Transform: ARC SINE(SQUARE ROOT(Y))

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	Lab	1.3510				
2	3.1	1.4120	30.00	16.00	5.00	
3	6.25	1.3794	28.00	16.00	5.00	
4	12.5	0.5923	15.00	16.00	5.00	*
5	25	0.1914	15.00	16.00	5.00	*
6	50	0.1588	15.00	16.00	5.00	*

Critical values are 1 tailed (k = 5)

STUDY NO.: 10562

ASSAY START: 08/26/02

SPECIES: A. bahia

EXPOSURE:

72 hours

SAMPLE: CH2MHill American Samoa

The binomial test shows that 6.25 and 12.5 can be used as statistically sound, conservative 95 percent confidence limits because the actual confidence level associated with these limits is greater than 95 percent.

An approximate LC50 of 10.6826 is obtained by nonlinear interpolation between 6.25 and 12.5

-----RESULTS CALCULATED USING THE MOVING AVERAGE METHOD-----

Span	G	LC50	95 Percent Confidence Limits	
4	1.867195E-02	11.43436	10.05015	12.96518
3	2.152512E-02	11.37471	10.13058	12.91414
2	2.510173E-02	11.2926	10.21447	12.41939
1	.0600481	10.6826	9.778212	11.9566

All LC50 calculated using the Moving Average method may not be a very good estimate if the span is much less than the number of concentrations.

-----RESULTS CALCULATED USING THE PROBIT METHOD-----

Iterations	G	H	Chi-Square	Probability
	7.773012E-02	1	2.206272	.5307138

Slope = 7.092948
95 Percent Confidence Limits = 5.115425 and 9.07047

LC50 = 11.18858
95 Percent Confidence Limits = 10.10101 and 12.38541

-----RESULTS CALCULATED USING THE TRIMMED SPEARMAN-KARBER METHOD-----

Conc.	Dose = Ln (Conc)	Percent Dead	Monotonic Rel. Freq.	Trimmed Rel. Freq.
50	3.912023	100	1	1.125 *
5	3.218876	98	.98	1.1 *
2.5	2.525729	68	.68	.725
6.25	1.832581	2	.02	-9.999999E-02 *
2.1	1.131402	0	0	-.125 *

Alpha = 10 %

Groups trimmed and therefore not used in estimating LC50 are marked with an asterisk above.

LC50 = 10.74899

Estimated 95 Percent Confidence Limits

Lower: 9.607583

Upper: 12.02599

Variance estimate = 3.150509E-03

STUDY NO.: 10562 ASSAY START: 08/26/02

SPECIES: A. bahia EXPOSURE: 72 hours

SAMPLE: CH2MHill American Samoa

SUMMARY TABLE

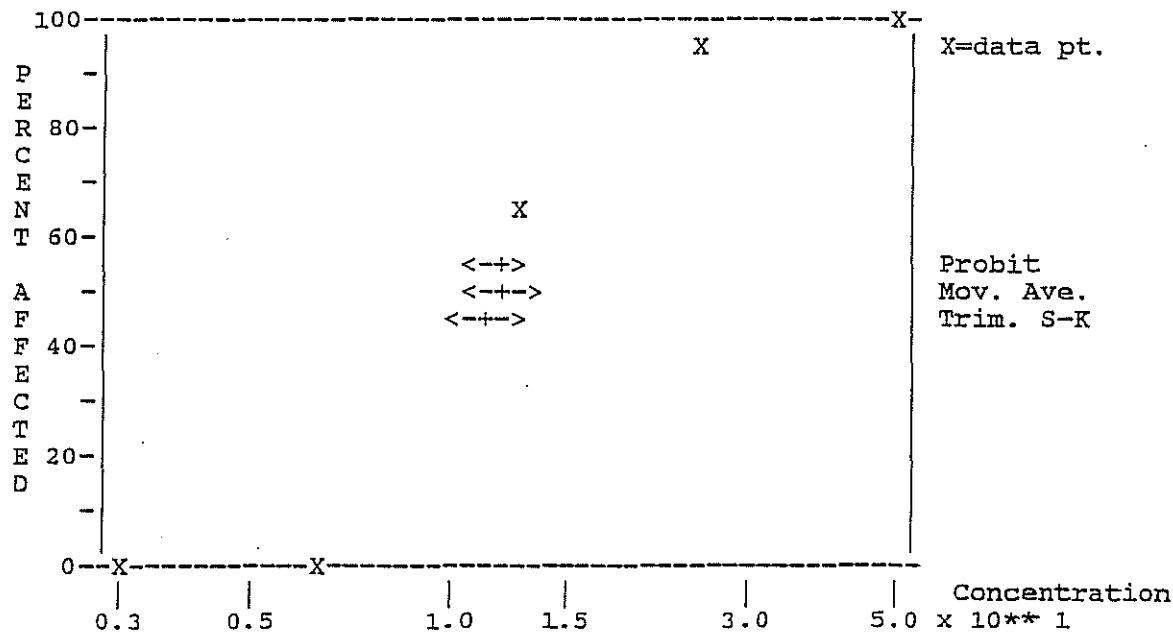
10-04-2002 10:53:44

Data:	Conc.	Exposed	Dead	Percent
	50	50	50	100
	25	50	49	98
	12.5	50	34	68
	6.25	50	1	2
	3.1	50	0	0

	LC50	Lower 95% Limit	Upper 95% Limit	
Probit Analysis	11.18858	10.10101	12.38541	
Moving Average	11.43436	10.05015	12.96518	Span= 4
Spearman-Kärber	10.74899	9.607583	12.02599	Alpha= 10 %

Binomial 10.6826

Compare results with original data to see if they are reasonable.



Title: 10562 American Samoa A. bahia Survival 96hrs
File: AmSamoa96 Transform: ARC SINE(SQUARE ROOT(Y))

Chi-Square Test for Normality

Actual and Expected Frequencies					
INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.0100	7.2600	11.4600	7.2600	2.0100
OBSERVED	3	4	18	3	2

Chi-Square = 8.1834 (p-value = 0.0851)

Critical Chi-Square = 13.277 (alpha = 0.01 , df = 4)
= 9.488 (alpha = 0.05 , df = 4)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: 10562 American Samoa A. bahia Survival 96hrs
File: AmSamoa96 Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's Test for Normality

D = 0.1791
W = 0.9593

Critical W = 0.9000 (alpha = 0.01 , N = 30)
W = 0.9270 (alpha = 0.05 , N = 30)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: 10562 American Samoa A. bahia Survival 96hrs
File: AmSamoa96 Transform: ARC SINE(SQUARE ROOT(Y))

Hartley's Test for Homogeneity of Variance
Bartlett's Test for Homogeneity of Variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

Title: 10562 American Samoa A. bahia Survival 96hrs
 File: AmSamoa96 Transform: ARC SINE(SQUARE ROOT(Y))

Summary Statistics on Transformed Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Lab	5	1.1071	1.4120	1.3185
2	3.1	5	1.2490	1.4120	1.3794
3	6.25	5	1.2490	1.4120	1.3794
4	12.5	5	0.4636	0.6847	0.5311
5	25	5	0.1588	0.3218	0.1914
6	50	5	0.1588	0.1588	0.1588

Title: 10562 American Samoa A. bahia Survival 96hrs
 File: AmSamoa96 Transform: ARC SINE(SQUARE ROOT(Y))

Summary Statistics on Transformed Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Lab	0.0189	0.1376	0.0615	10.4362
2	3.1	0.0053	0.0729	0.0326	5.2837
3	6.25	0.0053	0.0729	0.0326	5.2837
4	12.5	0.0099	0.0995	0.0445	18.7369
5	25	0.0053	0.0729	0.0326	38.0838
6	50	0.0000	0.0000	0.0000	0.0000

Title: 10562 American Samoa A. bahia Survival 96hrs
 File: AmSamoa96 Transform: ARC SINE(SQUARE ROOT(Y))

Steel's Many-One Rank Test - Ho: Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	Lab	1.3185				
2	3.1	1.3794	30.50	16.00	5.00	
3	6.25	1.3794	30.50	16.00	5.00	
4	12.5	0.5311	15.00	16.00	5.00	*
5	25	0.1914	15.00	16.00	5.00	*
6	50	0.1588	15.00	16.00	5.00	*

Critical values are 1 tailed (k = 5)

STUDY NO.: 10562 ASSAY START: 08/26/02

SPECIES: A. bahia EXPOSURE: 96hrs

SAMPLE: CH2MHill Am. Samoa Effluent

The binomial test shows that 6.25 and 12.5 can be used
is statistically sound, conservative 95 percent confidence limits
because the actual confidence level associated with these limits
is greater than 95 percent.

An approximate LC50 of 10.26096 is obtained by
nonlinear interpolation between 6.25 and 12.5

-----RESULTS CALCULATED USING THE MOVING AVERAGE METHOD-----

Span	G	LC50	95 Percent Confidence Limits	
4	2.157789E-02	10.69886	9.283869	12.24517
3	2.515953E-02	10.88285	9.613067	12.45337
2	2.510173E-02	10.88891	9.828579	11.97823
1	5.136475E-02	10.26096	9.471186	11.29766

An LC50 calculated using the Moving Average method may not be a very good
estimate if the span is much less than the number of concentrations.

-----RESULTS CALCULATED USING THE PROBIT METHOD-----

Iterations	G	H	Chi-Square	Probability
6	1.259855	7.704531	23.1136	LESS THAN 0.001

As the probability associated with this value is <0.05, results should
be used with caution.

Slope = 5.881265
95 Percent Confidence Limits = -.720059 and 12.48259

LC50 = 10.43415
95 Percent Confidence Limits = 0 and +INFINITY

-----RESULTS CALCULATED USING THE TRIMMED SPEARMAN-KARBER METHOD-----

Conc.	Dose = Ln (Conc)	Percent Dead	Monotonic Rel. Freq.	Trimmed Rel. Freq.
50	3.912023	100	1	1.125 *
25	3.218876	98	.98	1.1 *
12.5	2.525729	74	.74	.8
6.25	1.832581	2	.02	-9.999999E-02 *
3.1	1.131402	2	.02	-9.999999E-02 *

Alpha = 10 %

Groups trimmed and therefore not used in estimating LC50
are marked with an asterisk above.

LC50 = 10.23165

Estimated 95 Percent Confidence Limits

Lower: 9.226908

Upper: 11.3458

Variance estimate = 2.670923E-03

STUDY NO.: 10562 ASSAY START: 08/26/02

SPECIES: A. bahia EXPOSURE: 96hrs

SAMPLE: CH2MHill Am. Samoa Effluent

SUMMARY TABLE

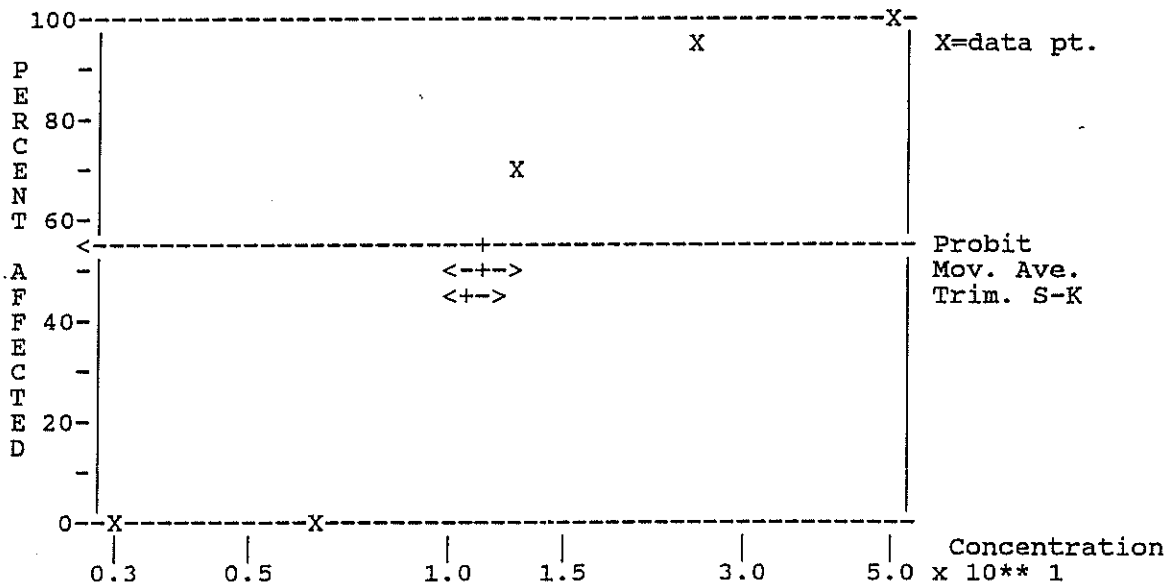
09-19-2002 10:01:58

Data:	Conc.	Exposed	Dead	Percent
	50	50	50	100
	25	50	49	98
	12.5	50	37	74
	6.25	50	1	2
	3.1	50	1	2

	LC50	Lower 95% Limit	Upper 95% Limit	
Probit Analysis	10.43415	0	0	
Moving Average	10.69886	9.283869	12.24517	Span= 4
Spearman-Kärber	10.23165	9.226908	11.3458	Alpha= 10 %

Binomial 10.26096

Compare results with original data to see if they are reasonable.



EnviroSystems, Incorporated
Organism Culture and Acclimation Data

I. Organism Information

Species: Americamysis bahia

Age: 1.5 days

Client: CH2M Hill

ESI#: 10562

Culturist: ESI

Date: 8/26/02

II. Culture Maintenance Information

Flow Through Recirc

By-pass Static

Brood Board

Spawning Renewal

Hatch Date: 8/24 20/02

Egg # 032602AB

Brood Organism A20

III. Acclimation History

Temperature: 23.2

Salinity: 29.7 ppt

Hardness: 100 mg/L CaCO3

pH: 7.56 SU

DO: 6.7 mg/L

Water Source: Natural Salt H₂O

SEE WATER ACCLIMATION (If applicable)

Start:

End:

PREPARATION OF DILUTIONS

STUDY: 10542		CLIENT: CH2M HILL - American Samoa						
SPECIES: <i>A. bahia</i>								
Diluent:	Day: 0		Day:					
Lab Salt	Sample: EOA		Sample: EOA					
Concentration	Vol. Eff.	Final Vol	Vol. Eff.	Final Vol	HRS	Date	Time	Initials
LAB	0	1000	0	750	0	8/26	1530	AP
3.1%	31	↓	23.3	↓	48	8/28	1630	RC
6.25%	62.5	↓	46.8	↓	Comments:			
12.5%	125	↓	93.75	↓				
25%	250	↓	37.5	150				
50%	500	↓	—	—				

RECORD OF METERS USED FOR WATER QUALITY MEASUREMENTS

STUDY: 10562		CLIENT: CH2M HILL - American Samoa				
WATER QUALITIES - A. bahia						
HOURS:	0	24	48 - old	48 - new	72	96
Water Quality Station #	1	2	1	2	2	2
Initials	AP	RC	RC	RC	PS	LM
Date	8/26	8/27	8/29	8/28	9/29/02	8/30

Water Quality Station #1		Water Quality Station #2		COMMENTS
DO meter #		DO meter #		
DO probe #	19	DO probe #	18	
pH meter #	1	pH probe #	2	
pH probe #	1097	pH meter #	50	
S/C meter #	5029	pH probe #	2830	
S/C probe #	YS130	S/C meter #	YS130	
Salinity meter #	↓	S/C probe #	↓	
		Salinity meter #	↓	

ESI

SAMPLE RECEIPT RECORD

EnviroSystems, Inc.
P.O. Box 778, One Lafayette Road
Hampton, N.H. 03843-0778
(603) 926-3345 • (603) 926-3521 Fax
E-mail ESI @ www.envirosystems.com

ESI STUDY NUMBER: 10562

SAMPLE RECEIPT DATE: 8/24/02 TIME: 1515

SAMPLE RECEIVED BY: AP

DELIVERED VIA: ☐ FEDEX ☐ CLIENT ☐ ESI ☐ UPS ☒ OTHER
DHL

SAMPLE CONDITION:

CHAIN OF CUSTODY: ☒ YES ☐ NO

CHAIN OF CUSTODY SIGNED: ☒ YES ☐ NO

CHAIN OF CUSTODY COMPLETE: ☒ YES ☐ NO

SAMPLE DATE: ☒ YES ☐ NO

SAMPLE TIME RECORDED: ☒ YES ☐ NO

SAMPLE TYPE IDENTIFIED: ☒ YES ☐ NO

CUSTODY SEAL IN PLACE: ☒ YES ☐ NO

SHIPPING CONTAINER INTACT: ☒ YES ☐ NO

SAMPLE TEMPERATURE (AT ARRIVAL): 17 °C

DOES CLIENT NEED NOTIFICATION OF TEMPERATURE?

☐ YES ☐ NO

SAMPLE ARRIVED ON ICE: ☐ YES ☒ NO

melted

COMMENTS:

☐ **CVO** 2300 NW Walnut Boulevard
Corvallis, OR 97330-3638
(541) 752-4271 FAX (541) 752-0276

Project # 147323.JC02.TW		Purchase Order #		Requested Analytical Method #										THIS AREA FOR LAB USE ONLY															
Project Name SAMOA JOINT CANNERY OUTFALL				TOTAL # OF CONTAINERS	<div style="display: flex; justify-content: space-between;"> <div style="writing-mode: vertical-rl; transform: rotate(180deg);">MYSID 96HE</div> <div style="writing-mode: vertical-rl; transform: rotate(180deg);">BUDASSAY</div> </div>										Lab #			Page			of								
Company Name CH2M HILL															Lab PM			Custody Review											
Project Manager or Contact & Phone # STEVE COSTA 707-677-0123															Report Copy to: SAME			Log In			LIMS Verification								
Requested Completion Date:			Site ID												Sample Disposal: Dispose <input checked="" type="checkbox"/> Return <input type="checkbox"/>			pH			Custody Seals Y N Ice Y N								
Sampling			Type												Matrix	CLIENT SAMPLE ID (9 CHARACTERS)										LAB QC	QC Level 1 2 3 Other		
Date	Time	COM P	GRA B	WATER	SOIL	AIR												Cooler Temperature											
8/23/02		X	X				02-TW										1 X	Alternate Description AERATE CONTINUOUSLY & BEFORE REPLENISHMENT										Lab ID	

DISTRIBUTION: Original - LAB, Yellow - LAB, Pink - Client



Steve Costa	216 Driftwood Lane	707-677-0123 (Vox)
Karen Glatzel	P.O. Box 1238	707-677-9210 (Fax)
	Trinidad, CA 95570-1238	510-508-5020 (Cell)

14 November 2002

Mr. Carl Goldstein
Pacific Insular Area Programs
CMD-1
Environmental Protection Agency
75 Hawthorne Street
San Francisco, CA 94105

Mr. Peter Peshut
American Samoa Environmental
Protection Agency
American Samoa Government
P.O. Box 368A
Pago Pago, American Samoa 96799

Enclosed is the required report for the bioassay test results for the August 2002 effluent sampling for the Joint Cannery Outfall in American Samoa. The sampling and analysis were carried out without problems. The result are similar to the past bioassay test results.

Please call us if you have any questions or comments on the enclosed report,

Sincerely,

A handwritten signature in blue ink that reads "Karen A. Glatzel".

Karen A. Glatzel

Cc: Jim Cox, COS International; Herman Gebauer, COS; Brett Ransby, COS;
John Brown, Heinz; Phil Thirkel, StarKist Samoa; Joe Carney, StarKist Samoa;
David Wilson, CH2M HILL.

Encl: Bioassay Testing - Joint Cannery Outfall Effluent August 2002 Sampling